

Nest-Site Selection in the Horseshoe Crab, *Limulus polyphemus*

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Abstract. Like a number of other species, horseshoe crabs lay their eggs on beaches in the intertidal zone. The elevation of the beach on which they nest differs among populations. We examined two factors that potentially affect egg survival at different beach elevations: erosion and rate of development. We found no evidence that eggs buried at different elevations incur different risks of erosion by wave action. However, the optimal beach elevation for egg development differed between our two study sites, Florida and Delaware, and the difference was related to beach morphology. Rate of development increased with oxygen concentration, redox potential, and temperature, and all three of these factors changed with elevation. The nests in the lower beach failed to develop, especially in Florida, where the beach was fine-grained and poorly drained. The nests in the upper beach were prone to desiccation, especially in Delaware, where the beach was course-grained and well-drained. This means that differences between sites in the optimal location for egg development coincided with horseshoe crab preferences in nest-site selection. We suggest that horseshoe crabs synchronize their nesting with the tides that reach the aerobic sediments on the beach, resulting in nesting patterns that differ with differences in tidal regimes and beach morphology.

Introduction

Horseshoe crabs (*Limulus polyphemus*) synchronize their spawning with flood tides, especially the highest (spring) tides associated with lunar syzygies (Lockwood, 1870; Rudloe, 1980; Cohen and Brockmann, 1983; Barlow *et al.*, 1986). Although they deposit their eggs in sediments found on the mid to upper beach, nesting elevation varies among populations (Shuster, 1982). For example, horseshoe crabs in Florida (Rudloe, 1980; Cohen and

Brockmann, 1983) and Massachusetts (Cavanaugh, 1975; Barlow *et al.*, 1986; Badgerow and Sydlik, 1989) nest in a narrow band in the upper middle quarter of the beach, whereas crabs in the Delaware Bay nest in a wide band over most of the wave-swept section of the beach (Shuster, 1982; Shuster and Botton, 1985; Botton *et al.*, 1992). The adaptive significance of nest-site preferences of horseshoe crabs is unclear, but it has been suggested that they nest at beach elevations where environmental conditions are most conducive to egg development (Lockwood, 1870; Shuster, 1982; Badgerow and Sydlik, 1989). Another possibility is that horseshoe crabs nest at elevations that minimize the loss of eggs due to beach erosion. In this paper we examine these hypotheses and the costs and benefits of the nest-site preferences of horseshoe crabs.

Horseshoe crabs spawn during the spring and early summer on certain beaches along the Atlantic and Gulf coasts of the United States, and in the Yucatan, Mexico (Shuster, 1982). At high tide females bury themselves in the sediments near the water's edge and lay a series of discrete egg clusters, each containing thousands of eggs (Brockmann, 1990). These eggs are fertilized by sperm released by an attached male and by one or more satellite males that typically congregate around the nesting couple (Rudloe, 1980; Brockmann, 1990; Brockmann and Penn, 1992; Brockmann *et al.*, 1994). The eggs develop in sediments 5 to 20 cm (mean 11.5 ± 2.8 SD cm) below the beach surface (Rudloe, 1979; Brockmann, 1990). After 2 to 4 weeks and four embryonic molts, the embryos hatch into "trilobite" larvae (Kingsley, 1892, 1893; Patten, 1896; Sekiguchi *et al.*, 1982; Sekiguchi, 1988). The nonfeeding larvae remain in the sand in distinct aggregations for several additional weeks, until they enter the ocean by moving toward the beach surface when tidal inundation occurs again (Rudloe, 1979). Within 2 weeks, the free-swimming trilobite larvae molt into juveniles that live in the near-shore sand flats (Sekiguchi *et al.*, 1982).

The preference that horseshoe crabs show for nesting in the upper elevations of the beach is puzzling because it is associated with several costs. (1) Individuals risk stranding on nesting beaches, which results in physiological stress or death from desiccation or predation (Botton and Loveland, 1989; Penn and Brockmann, in press). Stranding is riskier at higher beach elevations for two reasons: subsequent tides are less likely to "rescue" stranded crabs; and beach slope, which is necessary for orienting to the sea (Botton and Loveland, 1987; pers. obs.), diminishes in the upper beach. (2) Eggs located higher on the beach are likely to incur greater extremes of temperature and moisture and therefore may risk desiccation (Middaugh *et al.*, 1983). (3) Nesting on the beach requires that horseshoe crabs synchronize spawning with high tides, but this synchronization leaves individuals fewer opportunities to reproduce and increases competition for mates and nesting sites (Ims, 1990).

These costs suggest that there is some compensating benefit to the choice of spawning sites by horseshoe crabs. One possible explanation is that horseshoe crabs nest at elevations that optimize egg development (Lockwood, 1870; Badgerow and Sydlik, 1989). Shuster (1982) suggested that the development of *Limulus* eggs depends on a combination of temperature, moisture, and oxygen gradients on the beach, which vary according to local tidal amplitude. Sea turtles nest high on the beach because hatching success is increased by burying eggs in well-aerated sediments (Hays and Speakman, 1993; Horrocks and Scott, 1991). A second possible advantage is that wave action may be less likely to expose or wash away horseshoe crab eggs at certain beach elevations. Some intertidally spawning fishes, such as grunion, deposit their eggs in the upper intertidal to avoid beach erosion in the lower beach (Thompson, 1919; Taylor, 1984). To test hypotheses, we quantified the nesting locations of two populations of horseshoe crabs, Florida and Delaware, and conducted experiments that evaluated the effect of environmental variables and erosion on egg development and survival. The results of these experiments provide evidence for the adaptiveness of nest-site selection and spawning synchrony in horseshoe crabs.

Materials and Methods

Study sites

We studied horseshoe crabs at two locations in the United States: Florida (25 May to 5 June 1990 and 27 March to 31 April 1991) and Delaware (13 to 23 July 1990 and 11 May to 15 June 1991). The Florida (FL) site was a low-energy sandy beach on the south shore of Seahorse Key, a small island in the Gulf of Mexico, 4 km from Cedar Key (29°06' N, 83°04' W), Levy County. The Delaware site (DE) was a low-energy beach

of mixed sand and gravel located just inside the mouth of the Delaware Bay at the Cape Henlopen State Park (38°47' N, 75°06' W).

Beach measurements

To quantify beach elevation, we measured the oblique distance from a zero point on the beach to locations higher on the beach ("Beach Distance"). The zero point was the bottom of the beach where the mud flat ended and the beach began; *i.e.*, the slope and sediment composition changed abruptly. Beach distance was more practical to measure than elevation and the variations in beach slope, 2–5°, were insufficient to affect our measurements. We quantified the location at which eggs were laid by placing wire flagging stakes on either side of the female during nesting. We marked each egg cluster that the female laid by putting these flags across the female's hinge each time she moved forward in the sand; this procedure minimized the possibility of eggs from other females contaminating the egg clusters (Brockmann, 1990). At low tide we measured the distance from the bottom of the beach to the center of each set of flags ("Nesting Distance"). We also measured the water depth in which females nested and their distances to the water's edge. We quantified the timing of nesting by counting the number of nesting couples on the beach on three tides for 3 hours before and after the MHT in FL (1989) and DE (1991). "Tidal Distance" was quantified by measuring the extent of tidal inundation on the beach, using wooden stakes that were driven into the beach at 2-m intervals (measured as beach distances). We used tidal data recorded by the National Oceanic and Atmospheric Administration (at Cedar Key, FL, and Lewes, DE) to calculate mean high tide line (MHTL) for each. NOAA tide heights and our tidal distance measurements were highly correlated (FL $r = 0.92$; DE $r = 0.93$), which enabled us to interpolate the MHTL ($\pm 95\%$ CI) in tidal distance for April in FL and May in DE, 1991.

Egg development

To test the hypothesis that egg development was affected by beach elevation, we reburied newly laid eggs at three different beach distances and examined the eggs after 10 days (1990). After marking nests, we excavated and collected the freshly laid eggs at low tide from 25 (FL) and 22 (DE) different nests, broke up the clusters, separated the eggs from the sediments, and measured their volume (Brockmann, 1990). To separate the eggs we used a sieve (1 mm mesh) in FL, but the mixed sand-gravel beach in DE required that we elutriate the eggs from the sediments. We reburied 60 (FL) and 90 (DE) egg clusters ("Egg Batches") 8 cm below the sand surface at three different beach distances (3, 5, and 8 m) at 20 (FL) and 30 (DE) locations along 1 km of beach, marking their location with

flagging stakes. To prevent egg loss or contamination from other nests, half of the batches were placed in "cages" (2 mm nylon mesh bags sealed with Velcro). After 10 days we excavated the egg batches and quantified their development in two ways. First, we estimated the percent of developed eggs by visually inspecting the developmental stages of a 1-ml sample of eggs under a dissecting microscope, separating undeveloped eggs from embryos (*i.e.*, those with limb buds which occur during stage 13; Sekiguchi *et al.*, 1982), and counting the number to obtain a "Percent Eggs Developed" within each batch. Second, we measured the increase in the volume of egg batches ("Egg Batch Volume") due to developmental swelling (*i.e.*, the 1.7-mm *Limulus* eggs become 3.6-mm embryos before hatching). For example, volume of egg batches (due to developmental swelling) increased significantly with developmental rate (developmental index) ($r^2 = 0.65$, $F_{1,8} = 10.6$, $P = 0.01$). Changes in volume were calculated by dividing the volume at 10 days by the initial volume of an egg batch (V_2/V_1) because the initial volume of the egg batches varied (20–50 ml). Variation in initial volume of egg batches did not significantly affect developmental rate ($\beta = 0.25$, $T = 1.9$, $P = 0.06$).

To test the hypothesis that egg development was affected by environmental variables that changed with beach elevation, we collected freshly laid eggs from 13 (FL) and 23 (DE) nests and reburied the caged egg batches at 8 (FL) and 7 (DE) different predetermined beach distances for 12 days (1991). In DE we anchored the cages with flagging stakes so they would not be exposed by other nesting crabs. To quantify the average development of each egg batch after 12 days, we used a "Developmental Index" (D.I.). This index provided more resolution than "percent developed" used in 1990 because it weights development by the time required to reach each stage under standard conditions. Samples from each egg batch were divided into four categories based on developmental stages: eggs (stages 1–17), embryos (stages 18–19), late embryos (stages 20–21), and larvae (Sekiguchi *et al.*, 1982). After counting the number of individuals in each category, we multiplied this number by the number of days required to reach that stage in the laboratory (stage \times days) (Brown and Clapper, 1981). We calculated an overall D.I. for each batch by taking the sum of the stage \times days for all developmental stages divided by the total number of individuals. For example, the D.I. for one batch that had 5 eggs, 14 early embryos, 198 late embryos, and 0 larvae is $\sum[\text{stage} \times \text{days}] = [5(0) + 14(15) + 198(21) + 0(26)]/217 = 20$, which means that the batch contains a large proportion of late embryos. We used stepwise multiple regressions to determine the environmental factors that best predicted developmental rate.

We measured six environmental variables near the developing egg batches. (1) Temperature was measured *in*

situ with a thermistor probe inserted 10 cm below the surface, and measurements were taken three (DE) or five (FL) times for each egg batch (means used in analyses). (2) Moisture content (%H₂O) was measured by collecting sediment samples near each egg batch (in sealed plastic containers), weighing (within 2 h), drying (oven baked at 105°C), and reweighing (%H₂O = [(wet weight – dry weight)/wet weight] \times 100). (3) Salinity of the interstitial water (10 cm from surface) was measured in FL with a refractometer. (4) Interstitial oxygen concentration ([O₂]) could not be measured directly because %H₂O varies at different beach distances. Instead we used a portable YSI oxygen meter to measure the dissolved [O₂] in 120 ml of seawater inside a plastic bag that we buried in the sediments near the egg batches. Dissolved [O₂] within the bags equilibrates with the surrounding [O₂] sediments because polyethylene is permeable to oxygen (Fremling and Evans, 1963). Two (FL) or three (DE) measurements per bag were taken (mean used in analyses). We also collected sediments (50 ml) near each egg batch, added seawater (50 ml) to each sample, and then directly measured the [O₂] 1 h later at 30°C ("Sediment Analysis"). (5) Redox potential (Eh) was also measured inside the bags using a Fisher 955 pH/mV meter with a naked platinum electrode (Corning 476080 Redox Combination Electrode) because [Eh] is a more reliable indicator of oxygen availability than [O₂] measurements (McLachlan, 1978). [Eh] also indicates ion imbalances in sediments due to microbial activity (ZoBell, 1946; Bångér and Niemistö, 1978). (6) Sediment data were taken because the grain size determines the drainage and interstitial oxygen content of beaches (Eagle, 1983). Sediments were collected in FL and the mean grain size (M_z) and the variation, or sorting coefficient (σ_s), was measured by sieving (at 1ϕ intervals in the range -2 to 5ϕ). The units are on the phi (ϕ) scale, where $\phi = -\log_2 S$ and S is the grain size in mm (Boggs, 1987). The results were compared with descriptions of sediments from DE (Maurmeyer, 1978).

Because *Limulus* embryos can postpone development under anaerobic conditions (Palumbi and Johnson, 1982), we determined whether undeveloped eggs were still viable by transferring them to aerobic conditions in the laboratory. Eggs from each egg batch were reared in petri dishes (100 eggs per dish) with filtered seawater (periodically changed) in a FormaScientific incubator (under a 14 h daylight schedule at 30°C day, 26°C nighttime temperature; Brown and Clapper, 1981). In DE we noted the number of individuals within egg batches that were obviously dead (decayed and crumbly) after 12 days on the beach and compared the relative mortality of eggs at different beach distances.

Egg loss due to erosion

To test the hypothesis that beach elevation affects erosion of eggs from the sand, we measured the net amount

of sand lost (erosion) or deposited at different beach distances (1991). We hammered forty 1-m wooden stakes into the sand (the same stakes we used for measuring tidal distance). After the tide receded, we recorded the change in beach level ("erosion/deposition") that occurred since the previous tide. In FL we recorded the change in sand erosion/deposition at four beach distances ($n = 7$) using the mean from 11 high tides. In DE, we recorded the mean erosion/deposition at five beach distances ($n = 5$) from five tides. We looked for evidence of egg loss by comparing the change in volume between matched pairs of caged and uncaged egg batches from the egg development experiment (1990). Reduced volume in uncaged egg batches relative to caged ones would provide evidence for egg loss due to erosion. We also used egg-sized, colored glass beads to minimize the effects of predation (FL 1991). We made 30 "bead batches" (3-g batches), and buried them at three different beach distances, 8 cm below the surface ($n = 10$). To monitor the erosion during the experiment, we buried each batch near an erosion stake (using the mean erosion/deposition for analyses). After 17 days, the depth of the bead batches (the distance from the surface) was remeasured, and, after allowing them to dry, each batch was reweighed to estimate bead loss.

Data analyses

Before performing statistical tests, we examined the residual plots to validate the assumptions of the tests and used arcsine transformations on percentage data (to normalize variances). We used higher-level regression models (such as quadratic versus linear) only if there was a significant increase in the variation explained by adding an additional term to the model ($\alpha = 0.05$). We used multiple regressions to evaluate the significance of various factors and stepwise regression procedures to select the model when colinearity occurred. We used "Statview" (Abacus concepts, 1989a) and "SuperANOVA" (Abacus Concepts, 1989b) statistical software. Data are reported as mean \pm standard deviation, unless otherwise stated.

Results

Differences in nest-site location, tidal synchrony, and beach characteristics

Horseshoe crabs in DE nested higher and over a wider range of beach than those in FL (Fig. 1); the mean nesting distance and the variance were significantly greater in DE than in FL (Table I). In DE 95% of the crabs nested over 61% of the beach, whereas in FL 95% of the horseshoe crabs nested over 40% of the beach. The mean nesting distances were not significantly different from the mean high tide line (MHTL) in FL or DE (t -test, $P > 0.1$) and the nesting distances increased slightly with increasing

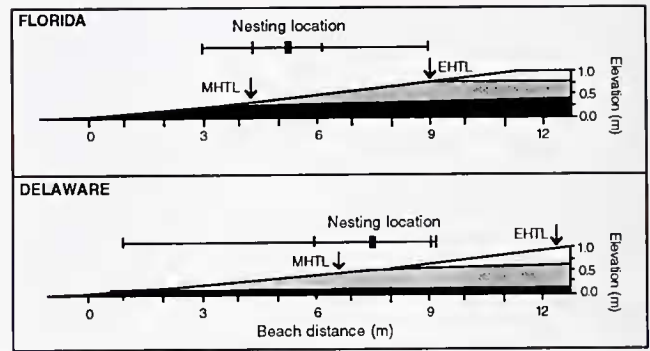


Figure 1. A comparison of the location on the beaches where horseshoe crabs nest in Florida and Delaware. "Nesting location" is the beach distance (mean \pm SD and range) where nesting occurs. MHTL is the mean high tide line and EHTL is the extreme high tide line. The moisture gradients on the beaches are shown as the saturated zone (■), moist zone (□), and drying zone (□).

tidal distance (FL: $r^2 = 0.25$, $F_{1,142} = 433$, $P = 0.0001$). The crabs nested in or just below the swash zone and neither the distance from the water's edge nor the water depth (t -test, $T_{179} = 1.1$, $P = 0.29$) of nesting crabs differed significantly between FL and DE populations (Table I).

The DE and FL populations exhibited different patterns of lunar and tidal synchrony (Table I). (1) In FL the crabs spawned only during spring tides. In DE the largest populations emerged during spring tides, but they continued to spawn during neap tides. (2) Spawning crabs were more abundant during the higher of the two daily tides in DE and FL. We noted one exception in DE when tidal inequality was reversing; *i.e.*, during lunar quadrature, the crabs were more abundant on the lower tide of the day in DE (see also Barlow *et al.*, 1986). In FL the highest tides were usually during the day, whereas in DE they were usually at night (1990; Table I). (3) In FL the largest numbers of horseshoe crabs nested around the time of the MHT (see also Cohen and Brockmann, 1983), but in DE they nested after MHT, *i.e.*, during the receding tide (Fig. 2).

The FL and DE beaches differed in a number of important respects (Table I). First, the extent of tidal inundation was greater in DE than in FL. Second, although both beaches can be classified as low energy, the FL beach was of a much finer grain size than the DE beach. Third, associated with these two variables was the fact that the saturated zone and the drying zones were higher on the beach in FL than in DE; *i.e.*, the finer grained sediments of the FL beach held water better than the coarser grained sediments of DE. Fourth, FL and DE beaches differed in the beach distances at which there was available oxygen (Table I).

Egg development

FL and DE differed in the pattern of egg development with beach distance. In FL, egg batches placed at the high-

Table 1

Nest-site selection by horseshoe crabs, the development of their eggs, and characteristics of the beach at various elevations at two sites, Florida and Delaware

	Florida	Delaware
Nesting Behavior		
<i>Location of Nesting Crabs</i>		
Mean nesting distance	5.1 ± 0.9 m (n = 434)	7.4 ± 1.9 m (n = 1396)
Range of nesting distance	3 to 9 m (n = 433)	1 to 9 m (n = 1395)
Mean distance from water's edge	68 ± 68 cm (n = 37)	85 ± 70 cm (n = 145)
Mean water depth	8 ± 9 cm (n = 37)	7 ± 8 cm (n = 144)
Nest depth	11.5 ± 2.8 cm (n = 382)	9.3 ± 3.9 cm (n = 112)
<i>Spawning Synchrony</i>		
Lunar	spring tides	spring and neap tides
Tidal	both daily tides (n = 13)	higher tide (n = 13) which usually occurs at night
Timing of maximum crab density	around high tide	after high tide
Egg Development (Percent Developed, 1991)		
<i>Beach Distance</i>		
11-12 m	Y	73
9-10 m	Y	74
7-8 m	81	97
5-6 m	70	95
3-4 m	40	76
0-2 m	0	39
Beach Characteristics		
<i>Tides</i>		
Tidal amplitude [†]	1.0 ± 0.5 m	2.0 ± 1.0 m
Mean high tide line	4.3 ± 0.6 m	6.7 ± 0.05 m
Extreme high tide line beach distance	9.0 m	12.5 m
Diurnal equality	nearly equal	unequal
Beach slope	2 to 5°	2 to 5°
<i>Beach Sediments</i>		
Percent gravel	0.0	0.1 to 82.0*
Percent sand	100	18.0 to 99.9*
Mean grain sizes (M _Z)	medium sand (0.31 mm, 1.67φ)	very coarse sand (1 mm, 0.008φ)*
<i>Moisture Gradient (at different beach distances)</i>		
Drying zone (<5%)	>8 m	≥4 m
Moist zone (5-17%)	3-8 m	1-3 m
Saturated zone (>18%)	<3 m	≤0 m
<i>Oxygen/Eh Gradient (at different beach distances)</i>		
High (>2.5 ppm and +Eh)	>5 m	>3 m
Medium (1-2 ppm and +Eh)	3-8 m	1-3 m
Low (<1 ppm and -Eh)	<3 m	≤0 m

[†] The predicted mean spring (and neap) tidal amplitudes (NOAA); ^Y Elevations above EHTL were not sampled; *Delaware data from Maurmeyer, 1978 (mean of four samples from the Breakwater Harbor beach face).

est location on the beach (8 m) were well-developed after 10 (1990; Table II) and 12 days (1991; Table I), whereas those placed at the lowest beach distances showed no signs of development and 26% were black, crumbly, and putrid smelling (1990; n = 14). Developmental rate (Fig. 3a) and egg-batch volume (Fig. 3b) increased significantly with beach distance. In DE the developmental rate of egg batches did not vary significantly with beach distance in 1990 (Table II), but in 1991 (Table I) developmental rate was maximal in the mid-section of the beach. Develop-

mental rate increased with beach distance up to 8 m, and then decreased (Fig. 3c), and egg-batch volume increased with beach distance up to 4 m, and then decreased (Fig. 3d). The greatest egg mortality occurred below 4 m and above 8 m ($r^2 = 0.15$, $P = 0.04$).

Oxygen concentration, redox potential, temperature, and moisture content varied significantly with beach distance in both FL and DE (Fig. 4). In FL developmental rate increased with all variables except salinity, which was invariably 26 ppt (n = 10), and these variables were

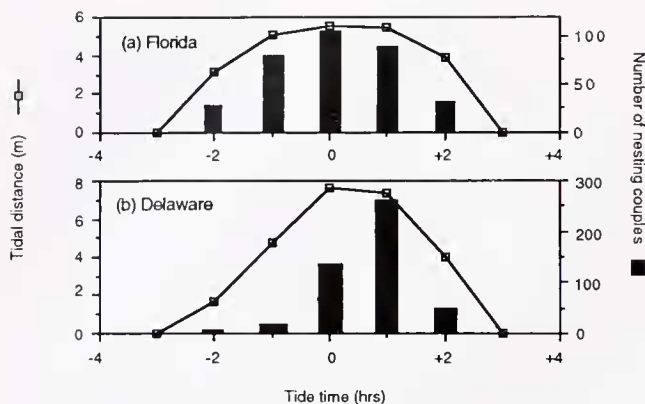


Figure 2. The time of maximum horseshoe crab spawning compared with the time of maximum high tide at (a) Seahorse Key, Florida (1989) and (b) Cape Henlopen, Delaware (1991). The bars indicate the mean number of nesting couples over three days and the points indicate the mean tidal distance recorded during three flood tides. "Tide time" indicates the hours before (-) and after (+) the maximum high tide (adapted from Burger *et al.*, 1977). Tidal synchrony was significantly different between the two populations ($\chi^2 = 945$, $df = 4$, $P < 0.001$).

intercorrelated with each other (Table III). A stepwise regression analysis found that $[O_2]$ was the variable that best predicted the developmental index in FL ($r^2 = 0.53$, $F_{1,22} = 25.2$, $P = 0.0001$), whereas the best predictor of D.I. in DE was $\%H_2O$ ($r^2 = 0.67$, $F_{2,23} = 23.6$, $P = 0.0001$).

The $[O_2]$ in the sediment samples (sediment analysis) increased significantly with beach distance ($r^2 = 0.45$, $F_{1,22} = 18$, $P = 0.0001$) in FL. Sand color below the surface was lighter at higher beach distances (ANOVA, $F_{1,158} = 143$, $P = 0.0001$) and was black or grey from 0 to 3 m, indicating anaerobic conditions (Eagle, 1983). Developmental rate (D.I.) of eggs in the incubator increased significantly with beach distance ($r^2 = 0.33$, $F_{1,34} = 16.7$, $P = 0.0003$) and eggs that had been buried in the lower beach (below 3 m) never hatched. Unlike FL, the sediments beneath the surface in DE were rarely grey or black, and most eggs in the lower beach showed some signs of development.

Egg loss due to erosion

In FL sand was eroded from the lower part of the beach and deposited at higher beach distances (Fig. 5). However, in the artificial egg experiment, bead loss occurred only at the highest beach distance (ANCOVA, $F_{1,28} = 7.92$, $P = 0.009$). No significant associations were found between bead loss ($P = 0.75$) or final bead depth ($P = 0.70$) and sand erosion. In the egg-loss experiment, volume (V_2/V_1) did not differ between caged and uncaged treatments (Wilcoxon Signed-Ranks, $z = 0.77$, $n = 13$, $P = 0.22$). In DE sand was eroded only at the highest beach distance ($r^2 = 0.23$, $F_{1,22} = 6.9$, $P = 0.02$) and this was negligible

(i.e., < 5 cm) (Fig. 5). Egg batch volume (V_2/V_1) did not differ between caged and uncaged treatments ($F_{1,74} = 2.7$, $P = 0.12$).

Discussion

Nest-site selection and egg development

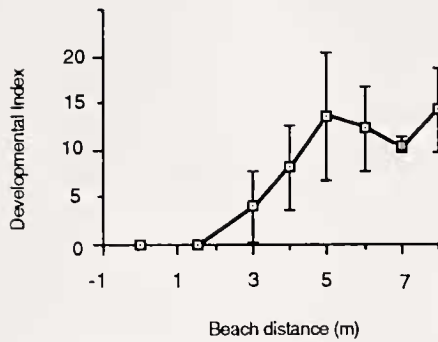
Horseshoe crabs nested at beach elevations where egg development was maximized. They nested just above the mean high tide line, avoiding the lower and upper elevations where egg development was impaired. Our results show that variation in egg development with elevation is due to chemical and thermal gradients on the beach. The sediments in the upper beach were warmer and drier than the lower beach, which explains why eggs buried above the mean tide line were more likely to desiccate than eggs buried lower on the beach. The sediments in the lower beach contained insufficient interstitial oxygen concentrations for egg development to occur. The color and odor of the lower beach indicated the presence of hydrogen sulfide and explains why the eggs became black and crumbly. The inability of eggs to survive in anoxic sediments supports the suggestion that nesting horseshoe crabs avoid beaches with anoxic peat beds because they impair embryonic development (Botton *et al.*, 1988). Horseshoe crabs may avoid anaerobic conditions (anoxic sediments, peat beds, and sewer outflows; Rudloe, 1971) by using oxygen-sensitive elements in their book gills (Crabtree and Page, 1974) and prosoma (Thompson and Page, 1975). It has also been suggested that horseshoe crabs have H_2S receptors (Botton *et al.*, 1988).

Horseshoe crabs in Delaware nest over a wider range of the beach than in Florida, probably because the range of elevations conducive to development is wider in Delaware. The differences in egg development between the two sites can be explained by differences in oxygen and moisture gradients on the beaches. In Florida the interstitial oxygen concentration increased slowly with distance (not reaching $[O_2] > 1$ ppm and $+Eh$ until 3 m), whereas oxygen concentration increased sharply with beach distance in Delaware ($[O_2] > 1$ ppm and $Eh > +80$ above 1 m section of the beach) (Figs. 4a, e). Also the redox potentials in the lower beach in Delaware ($+140$ to -30 mV) were never as low as in Florida (-50 to -250 mV) (Figs. 4b, f). This means that the Delaware crabs can nest lower on the beach without adversely affecting the development of their eggs.

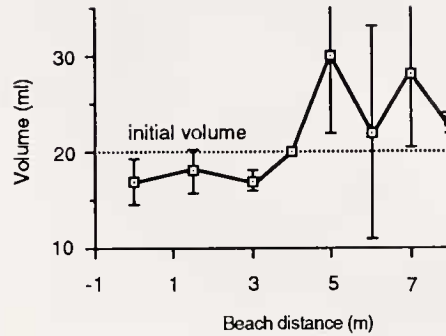
The differences in interstitial oxygen (and redox gradients) and moisture gradients between the Florida and Delaware sites can ultimately be attributed to differences in beach morphology (Table 1). Sediment grain size determines the drainage of a beach, which greatly affects the interstitial oxygen content (Gordon, 1960; Brafield, 1964; Eagle, 1983). The Florida sediments were fine to medium

FLORIDA

(a) Egg Development

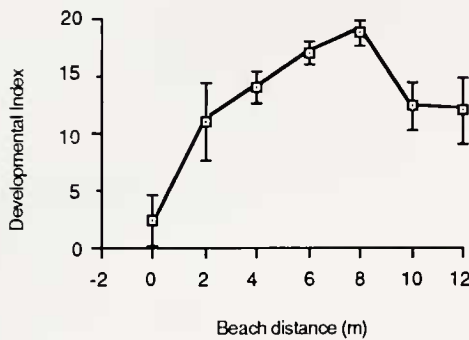


(b) Egg-Batch Volume



DELAWARE

(c) Egg Development



(d) Egg-Batch Volume

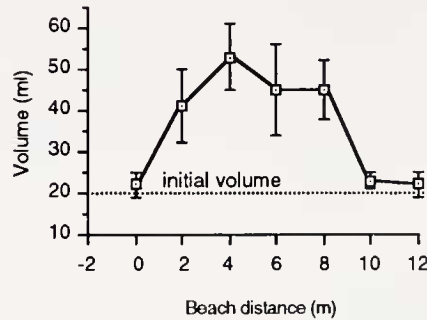


Figure 3. Development of *Limulus* eggs, embryos and larvae (mean \pm SE) at different beach distances in Florida (a and b) and Delaware (c and d). Development, as measured by the developmental index, varied with beach distance in (a) Florida ($r^2 = 0.35$, $F_{1,21} = 11.3$, $n = 3$, $P = 0.003$) and in (c) Delaware ($r^2 = 0.44$, $F_{2,42} = 16$, $n = 7$, $P = 0.0001$). Egg batch volume due to developmental swelling also varied with beach distance in (b) Florida ($r^2 = 0.12$, $F_{1,21} = 2.9$, $P = 0.10$) and (d) Delaware ($r^2 = 0.32$, $F_{2,42} = 9.7$, $P = 0.0003$).

grained and had poor drainage (e.g., the moisture content decreased gradually, not dropping to 5% until 7 m), whereas the Delaware sediments were coarse grained with relatively high drainage (e.g., the interstitial water dropped sharply, falling below 5% at 4 m) (Figs. 4d, and h). Thus, even though the tidal amplitudes were greater in Delaware, the water content of the beach was lower than in Florida. Fine-grained sediments, such as in Florida, also have greater surface areas for microbial growth. This further depletes interstitial oxygen, increases hydrogen sulfide, and lowers redox levels (Eagle, 1983; Boaden, 1985). Variation in beach geochemistry may also explain why the crabs in Florida nested up to the EHTL but well below this mark in Delaware (Fig. 1). In Delaware the eggs in the highest part of the beach were usually desiccated. Water retention was so poor in Delaware that water concentration was the best predictor of egg development on the

beach. Increased risk of desiccation on higher parts of the beach may explain why crabs in Massachusetts shift their nesting sites to lower beach distances during the summer (Barlow *et al.*, 1986).

Nesting synchrony

Nesting synchrony varied significantly between the two populations. In the Delaware Bay, horseshoe crabs often spawn during neap tides, as reported for Massachusetts (Cavanaugh, 1975; Barlow *et al.*, 1986; pers. obs.). However, crabs in Florida almost never spawn during neap tides (Rudloe, 1980; Cohen and Brockmann, 1983; pers. obs.). Our results from the egg-development experiments suggest an adaptive explanation for differences in spawning synchrony between these populations. The aerobic sediments occur at higher elevations in Florida than in

Table II

The development of *Limulus* eggs placed at different locations on the beach

Beach distance	Florida		Delaware	
	% Developed	<i>n</i>	% Developed	<i>n</i>
Upper beach (8 m)	67 ± 32	23	61 ± 33	30
Middle beach (5 m)	5 ± 2	21	65 ± 30	29
Lower beach (3 m)	0	14	59 ± 33	27

Results from the 1990 development experiment. Egg development (percent eggs developed $\bar{x} \pm$ SD) increased with beach distance in Florida (ANOVA, $F_{2,55} = 82$, $P = 0.0001$), but not in DE ($F_{2,78} = 0.39$, $P = 0.68$). Overall, egg development was significantly greater in DE than in FL ($F_{1,142} = 886$, $P = 0.0001$).

Delaware, and neap tides are lower in Florida than in Delaware (Table I; tidal amplitude decreases with latitude in the eastern U.S.). In Florida the flood tides rarely reach the aerobic zone of the beach (Fig. 6), which explains why horseshoe crabs there do not nest during neap tides.

Nesting synchrony corresponds to local tidal patterns in yet another way. In Florida the two daily flood tides were nearly equal in height and the crabs spawned on both (Rudloe, 1980; Cohen and Brockmann, 1983). However, in northern latitudes, the two daily tides were disproportionate in size (indeed, the largest neap tides are higher than the lower spring tides) and northern horseshoe crabs preferred the higher of the two tides (Barlow *et al.*, 1986; pers. obs.), which occurred at night during the spring and early summer. Only the higher of the two tides at this latitude reached the aerobic zone of the beach (Fig. 6). Knowing the local tidal rhythms and the location of the aerobic zone on the beach may enable one to predict the location and timing of horseshoe crab spawning.

Another similarity in spawning synchrony between Delaware and Massachusetts populations, which differed from Florida, is that the crabs in both locations largely spawned one hour after the maximum high tide (Howard *et al.*, 1984). There are several possible explanations for nesting during the receding tide. (1) The mixed sand and gravel sediments in Delaware may make nest excavation relatively difficult (*e.g.*, the carapaces of females are often eroded in Delaware Bay). The crabs may reduce nesting effort by nesting during the receding tide when the sediments seem to be softer (pers. obs.). (2) Because the scour phase occurs on the incoming tide (whereas the deposition phase occurs on receding tides) (Strahler, 1966), nesting on the receding tide might minimize egg loss due to erosion. However, we had little erosion of eggs at any locality so this seems unlikely. (3) Nesting on the receding tide might minimize egg loss caused by disturbance from other nesting crabs, which appears to be the major source of egg loss in Delaware Bay (Barash, 1993; pers. obs.).

Nest-site selection and egg loss due to erosion

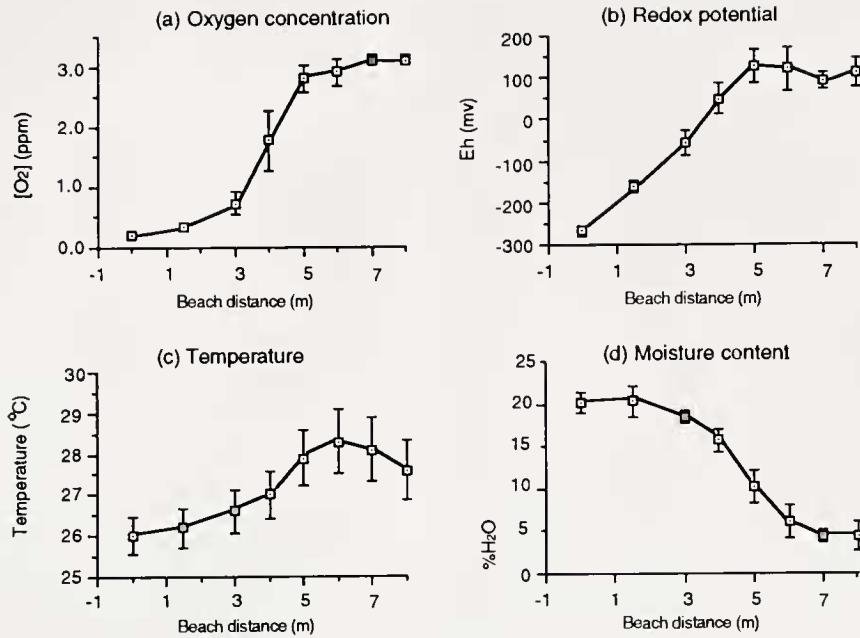
We found no evidence that nests at lower elevations were more likely to be washed away by erosion in Florida or Delaware. Our measurements indicated that erosion was insufficient to uncover horseshoe crab nests buried 5 to 20 cm below the sand surface (Brockmann, 1990). Measurements of erosion recorded during the incoming "scouring phase" of the tide, however, reveal that the lower beach is more dynamic than the upper beach (Strahler, 1966). Still, in the artificial egg experiment, more beads were lost in the upper beach (where wave action appeared to be the greatest). However, bead loss may have been due to nesting crabs uncovering beads, rather than erosion, because bead-depth and erosion did not vary with bead loss. Such differential excavation of beads by nesting crabs was not controlled in the experiment because the densities of spawning crabs were relatively low in Florida, and because caged controls interfere with erosion (Quammen, 1981). Despite the negative effect here, it must be noted that our experiments did not incur any storms at either locality, and substantial beach erosion occurs during severe storms (pers. obs.).

Nest-site selection and egg loss due to predation

Horseshoe crabs are generally thought to nest on beaches to minimize egg loss from aquatic predators such as fish (Rudloe, 1980; Cohen and Brockmann, 1983; Botton and Loveland, 1989). Eggs and larvae have been found in several fishes in Delaware Bay (Shuster, 1982). We observed small schools of striped killifish (*Fundulus majalis*) burrowing into nests that were located just below the sand surface in Florida, but only on four occasions in 1991. We found no differences in egg loss when we compared caged and uncaged eggs at any beach elevation in Florida or Delaware (Penn, 1992). We suspect that fish feed mainly on eggs uncovered by nesting crabs and wave action (Shuster, 1960), and on trilobite larvae that are moving into the sea at high tide (Rudloe, 1979). Even if aquatic egg predation is a negligible selective pressure on nest-site selection in horseshoe crabs today, aquatic egg predators may have provided the initial selective pressures for intertidal spawning in ancestral merostomes. Intertidal spawning may have preadapted the early chelicerates for the initial animal invasion of land (Størmer, 1977; Little, 1983).

The heaviest predation on horseshoe crab eggs is undoubtedly from shorebirds (Mallory and Schneider, 1979; Botton, 1984; Castro *et al.*, 1989; Castro and Myers, 1993; Botton *et al.*, 1994). Although shorebirds might be expected to feed on nests located higher on the beach, they often forage along the water's edge following the tide line (Recher, 1966; Recher and Recher, 1969; Burger *et al.*, 1977; Evans, 1988; pers. obs.) apparently capitalizing on

FLORIDA



DELAWARE

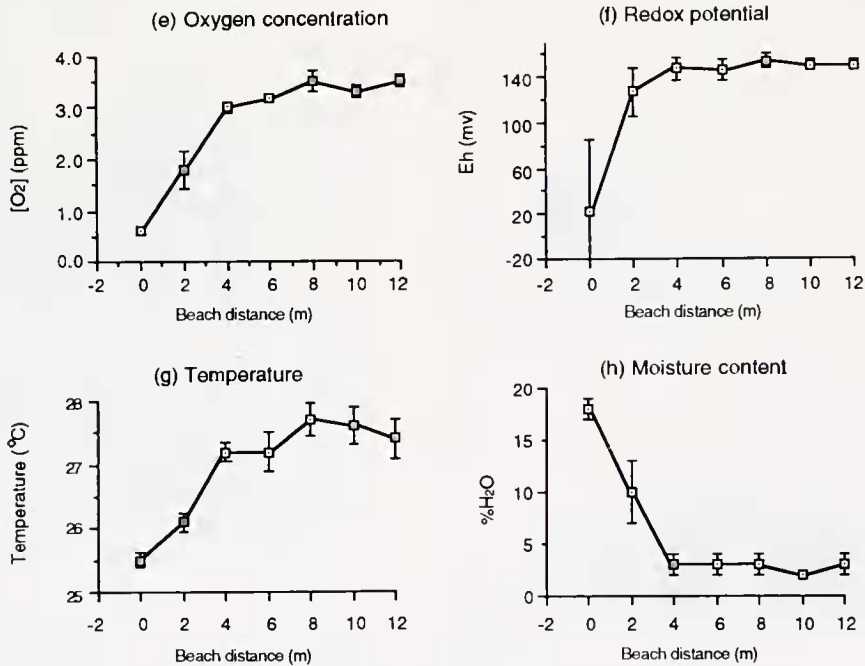


Figure 4. The conditions of the sediments at different beach distances (1991; mean \pm SE, $n = 3$) in Florida: (a) interstitial oxygen concentration ($r^2 = 0.78$; $F_{1,22} = 78$; $P = 0.0001$), (b) redox potential ($r^2 = 0.79$; $F_{1,19} = 70$; $P = 0.0001$), (c) temperature ($r^2 = 0.64$; $F_{1,22} = 39$; $P = 0.0001$), (d) moisture content ($r^2 = 0.80$; $F_{1,22} = 87$; $P = 0.0001$); and Delaware: (e) interstitial oxygen concentration ($r^2 = 0.82$; $F_{2,46} = 102$; $P = 0.0001$), (f) redox potential ($r^2 = 0.36$; $F_{2,39} = 11$; $P = 0.0002$), (g) temperature ($r^2 = 0.61$; $F_{2,46} = 35$; $P = 0.0001$), and (h) moisture content ($r^2 = 0.83$; $F_{2,27} = 67$; $P = 0.0001$).

Table III

The effects of four environmental factors in beach sediments on the development of *Limulus* eggs (D.I.) at the FL and DE sites (1991)

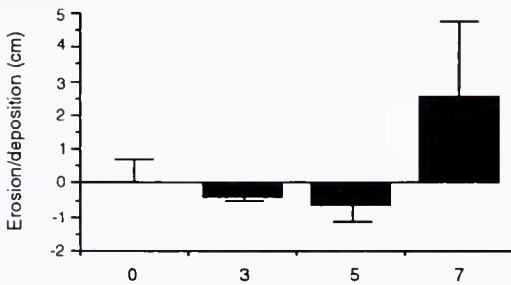
Parameter	Florida		Delaware	
	r^2	Significance test	r^2	Significance test
[O ₂]	0.53	$F_{1,22} = 25.2; P = 0.0001$	0.42	$F_{2,42} = 15; P = 0.0001$
Eh	0.32	$F_{1,19} = 8.8; P = 0.0008$	0.31	$F_{2,35} = 7.7; P = 0.002$
Temperature	0.44	$F_{1,22} = 17.6; P = 0.0004$	0.32	$F_{2,42} = 10.7; P = 0.0002$
%H ₂ O	0.41	$F_{1,22} = 15.2; P = 0.0001$	0.67	$F_{2,23} = 23.6; P = 0.0001$
[O ₂] and Eh	0.68	$F_{1,19} = 40; P = 0.0001$	0.33	$F_{1,40} = 19.6; P = 0.0001$
[O ₂] and temperature	0.76	$F_{1,22} = 70; P = 0.0001$	0.56	$F_{1,47} = 64; P = 0.0001$
[O ₂] and %H ₂ O	0.80	$F_{1,22} = 86.3; P = 0.0001$	0.92	$F_{1,26} = 251; P = 0.0001$
Temperature and Eh	0.49	$F_{1,19} = 18.1; P = 0.0004$	0.26	$F_{1,40} = 14; P = 0.0006$
Temperature and %H ₂ O	0.84	$F_{1,22} = 119; P = 0.0001$	0.58	$F_{1,20} = 30.2; P = 0.0001$
Eh and %H ₂ O	0.45	$F_{1,19} = 16; P = 0.0008$	0.43	$F_{1,21} = 16; P = 0.0007$

Note: A polynomial regression was used on the Delaware data and nonsignificant effects were not included.

the softer substrate at the water's edge. Because the tides rarely reach them, higher nests may actually be safer from the probing bills of shorebird predators than lower nests. Such "bottom-up" predation has been found in several species of shorebirds (Prater, 1972; Ambrose, 1986; Peterson, 1991). Our observations in Florida suggest that

most horseshoe crabs nest above the elevation where sanderlings (*Calidris alba*) and willets (*Catoptrophorus semipalmatus*) forage (Penn, 1992). This would favor avoiding nesting in the lower beach. In Delaware laughing gulls (*Larus atricilla*) and numerous shorebirds fed on *Limulus* eggs. However, rather than probing below the surface, the birds fed on the copious eggs on the beach

(a) FLORIDA



(b) DELAWARE

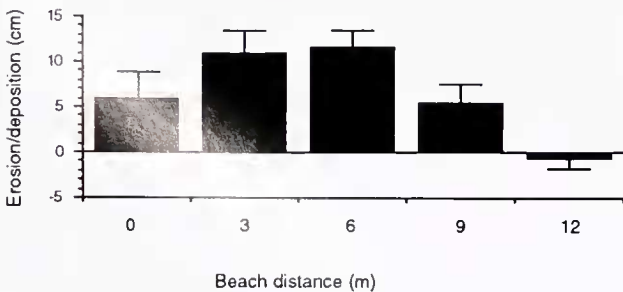


Figure 5. Changes in beach level from erosion and deposition of sediments at different beach distances in (a) Florida and (b) Delaware. Deposition occurred at the highest beach distance in Florida ($r^2 = 0.32; F_{1,26} = 12; P = 0.002$), whereas erosion occurred at the highest beach distance in (b) Delaware ($r^2 = 0.23; F_{1,22} = 7; P = 0.02$).

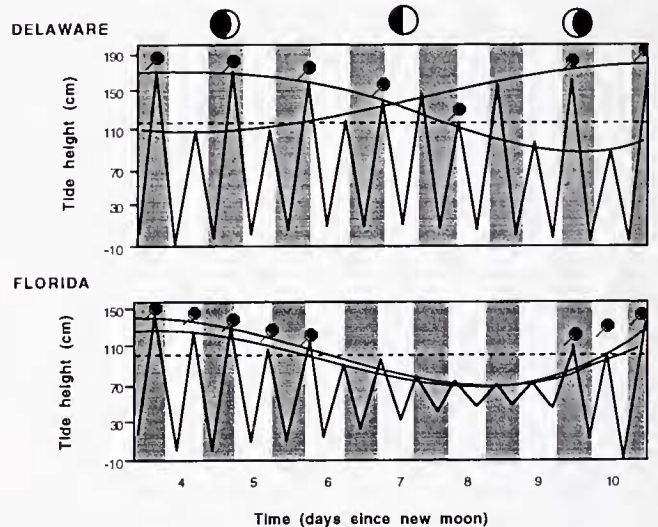


Figure 6. A model of spawning synchrony of horseshoe crabs with tidal rhythms in Delaware and Florida. Tidal amplitude (\sqrt{V}) decreases during lunar quadrature (neap tides) and increases during lunar syzygy (spring tides). Tidal inequality of daily tides (sinusoidal lines) is greatest during spring tides, especially in Delaware. Horseshoe crabs in Delaware synchronize their spawning with the higher of the two daily tides (which usually occurs at night), which enables them to reach the aerobic sediments on the beach (dashed lines). Horseshoe crabs in Florida do not spawn during neap tides, when the tides fail to reach the aerobic elevations. However, during spring tides they spawn during both daily tides as there is little tidal inequality.

surface uncovered by nesting crabs (Barash, 1993; Botton *et al.*, 1994). Thus, it seems unlikely that nest-site selection is an adaptation to protect eggs from shorebirds in Delaware.

Conclusions

Horseshoe crabs nest just above the mean high tide line where the development of their eggs is maximized. Beach sediments lower on the beach contain inadequate interstitial oxygen concentrations (and probably high H₂S), whereas sediments higher on the beach are too dry for egg development. Differences in nest-site preferences between the Florida and Delaware horseshoe crabs correspond to differences in the location where eggs successfully develop, which is ultimately due to differences in geochemistry on these beaches. Although beach geochemistry and local tidal rhythms provide a partial explanation for the geographic variation in nest-site preferences and nesting synchrony among horseshoe crabs, they do not explain all of the variation in nesting behavior. Other factors, such as predation and intraspecific competition for nesting sites, are likely to affect nest-site selection in *Limulus*.

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